

arginine and L-proline. More preferably, the present invention relates to the production of L-lysine.

[0040] The present invention provides an isolated or purified polypeptide encoded by the DNA plasmid encoding pyruvate carboxylase contained in Deposit Number NRRL B-30293, the amino acid sequence of SEQ ID NO:2 or the amino acid sequence of SEQ ID NO:4. Still another aspect of the present invention provides a polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16 and SEQ ID NO:18.

[0041] Accordingly, SEQ ID NO:6 corresponds to the amino acid sequence: PSKNIDDIVKSAE. SEQ ID NO:8 corresponds to the amino acid sequence: RGMRFVSSPDELK. SEQ ID NO:10 corresponds to the amino acid sequence: AAFGDSVYVERA. SEQ ID NO:12 corresponds to the amino acid sequence: VQILGDRTEVVH. SEQ ID NO:14 corresponds to the amino acid sequence: IATGFIGDHPHLL. SEQ ID NO:16 corresponds to the amino acid sequence: TITASVEGKIDRV. SEQ ID NO:18 corresponds to the amino acid sequence: MTAITLGGLLKGIITLV.

[0042] All of the polypeptides of the present invention are preferably provided in an isolated form. As used herein, "isolated polypeptide" is intended to mean a polypeptide removed from its native environment. Thus, a polypeptide produced and/or contained within a recombinant host cell is considered isolated for purposes of the present invention. Also intended as an "isolated polypeptide" are polypeptides that have been purified, partially or substantially, from a recombinant host. For example, a recombinantly produced version of the pyruvate carboxylase enzyme can be substantially purified by the one-step method described in Smith and Johnson, *Gene* 67:31-40 (1988).

[0043] One aspect of the present invention include the polypeptides which are at least 80% identical, more preferably at least 90%, 95% or 100% identical to the polypeptide encoded by the DNA plasmid encoding pyruvate carboxylase

contained in Deposit Number NRRL B-30293, the polypeptide of SEQ ID NO:2 or the polypeptide of SEQ ID NO:4.

[0044] By a polypeptide having an amino acid sequence at least, for example, 95% "identical" to the amino acid sequence of SEQ ID NO:2, for example, it is intended that the amino acid sequence of the polypeptide is identical to the reference sequence except that the polypeptide sequence may include up to five amino acid alterations per each 100 amino acids of the amino acid sequence of SEQ ID NO:2, for example. In other words, to obtain a polypeptide having an amino acid sequence at least 95% identical to a reference amino acid sequence, up to 5% of the amino acid residues in the reference sequence may be deleted or substituted with another amino acid, or a number of amino acids up to 5% of the total amino acid residues in the reference sequence may be inserted into the reference sequence. These alterations of the reference sequence may occur at the amino or carboxy terminal positions of the reference amino acid sequence or anywhere between those terminal positions, interspersed either individually among residues in the reference sequence or in one or more contiguous groups within the reference sequence.

[0045] As a practical matter, whether any particular polypeptide is, for instance, 95% identical to the amino acid sequence shown in SEQ ID NO:2, SEQ ID NO:4 or to the amino acid sequence encoded by deposited DNA clone can be determined conventionally using known computer programs such the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). When using Bestfit or any other sequence alignment program to determine whether a particular sequence is, for instance, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference amino acid sequence and that gaps in homology of up to 5% of the total number of amino acid residues in the reference sequence are allowed.

[0046] Another aspect of the present invention provides a nucleic acid molecule encoding the polypeptide comprising the amino acid sequence selected from the group consisting of SEQ ID NO:6, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:12, SEQ ID NO:14, SEQ ID NO:16 and SEQ ID NO:18. Preferably, the invention provides for nucleic acid molecules, which code for the aforementioned polypeptides, that are selected from the group consisting of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9, SEQ ID NO:11, SEQ ID NO:13, SEQ ID NO:15 and SEQ ID NO:17.

[0047] Accordingly, SEQ ID NO:5 corresponds to the nucleic acid sequence that codes for the amino acid sequence of SEQ ID NO:6. SEQ ID NO:7 corresponds to the nucleic acid sequence that codes for the amino acid sequence of SEQ ID NO:8. SEQ ID NO:9 corresponds to the nucleic acid sequence that codes for the amino acid sequence of SEQ ID NO:10. SEQ ID NO:11 corresponds to the nucleic acid sequence that codes for the amino acid sequence of SEQ ID NO:12. SEQ ID NO:13 corresponds to the nucleic acid sequence that codes for the amino acid sequence of SEQ ID NO:14. SEQ ID NO:15 corresponds to the nucleic acid sequence that codes for the amino acid sequence of SEQ ID NO:16. SEQ ID NO:17 corresponds to the nucleic acid sequence that codes for the amino acid sequence of SEQ ID NO:18.

[0048] Methods used and described herein are well known in the art and are more particularly described, for example, in J.H. Miller, *Experiments in Molecular Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1972); J.H. Miller, *A Short Course in Bacterial Genetics*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1992); M. Singer and P. Berg, *Genes & Genomes*, University Science Books, Mill Valley, California (1991); J. Sambrook, E.F. Fritsch and T. Maniatis, *Molecular Cloning: A Laboratory Manual*, 2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989); P.B. Kaufman *et al.*, *Handbook of Molecular and Cellular Methods in Biology and Medicine*, CRC Press, Boca Raton, Florida (1995); *Methods in*

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